Journal of
APPLIED PACKAGING RESEARCH

RESEARCH ARTICLE

Polyestyrene and Low Density Polyethylene Oregano's Essential Oil Functionalization for Possible Antimicrobial Active Packaging Applications

Edgar Díaz-Acosta*
Universidad Autónoma de Ciudad Juárez

Claudia Rodríguez-González
Universidad Autónoma de Ciudad Juárez

Laura Valencia-Gómez
Universidad Autónoma de Ciudad Juárez

Santos Adriana Martel-Estrada
Universidad Autónoma de Ciudad Juárez

Maria Hernández-González
Universidad Autónoma Agraria Antonio Narro

Hortensia Reyes-Blas
Universidad Autónoma de Ciudad Juárez

Imelda Olivas Armendáriz
Universidad Autónoma de Ciudad Juárez

ABSTRACT

Low density polyethylene (LDPE) and polystyrene (PS) samples were functionalized with oregano's essential oil (OEO) for possible antimicrobial packing applications. Different concentrations of OEO were used (0.5-1.5 % v/v). Polymers were UV light irradiated during 60 min to improve the OEO adhesion through the polar groups (COOH, OH or COO-) generation on their surface. The effect of OEO different concentration on their antimicrobial activity, mechanical properties and cytotoxicity of the functionalized polymers were analyzed. All functionalized samples showed antimicrobial activity against Gram positive and Gram negative bacteria and this activity increased with OEO concentration. The addition of OEO did not affect the LDPE mechanical properties. The flexural properties of PS were affected by the OEO highest concentration. A cytotoxic effect was found in samples with 1.0 % and 1.5 % v/v OEO concentrations.

KEY WORDS

Surface treatment, antimicrobial film, cytotoxicity, oregano essential

***Edgar Díaz-Acosta**
Corresponding Author
edgar.diaz@uacj.mx

INTRODUCTION

In recent years, polymers for food packaging have been widely used. These packages are used to maintain and protect food from the environmental effect and microbial spoilage. New active materials known as active packaging have been developed to improve fresh products preservation. Active packaging is defined as a material containing substances in its structure that interact with the packed food or the surrounding environment to extend the shelf life of perishable food [1]. The first antimicrobial package application to enhance the shelf life of red meat was achieved through the use of modified atmosphere packaging (MAP) technology, by eliminating the oxygen presence and increasing the carbon dioxide concentration to decrease the growth of aerobic microorganisms [2]. Pathogenic bacteria and those that spoil food are treated through chemical control; the use of synthetic chemical compounds is limited due to undesirable effects such as carcinogenicity, acute toxicity, teratogenicity and slow degradation time. This has raised an interest in the use of natural synthesized compounds [3]. Active package represents a promising alternative instead the use of antimicrobial substances applied directly to the food, the essential oils adsorption, from several plants, on polymeric surfaces has been proven as antioxidant and antimicrobial options [4]. Essential oils are effective against certain types of pathogenic bacteria such as *Escherichia coli (E. coli)*, *Salmonella Typhimurium, Staphylococcus aureus (S. aureus), Listeria Monocytogenes (L. Monocytogenes)* and *Campylobacter,* among others [5]. Oregano is an aromatic plant widely cultivated in Mediterranean countries, used to enhance flavor. The antimicrobial properties of its essential oil makes it a good alternative as natural food preservative [6]. The study of oregano essential oil (OEO) incorporated into polymeric films has raised in recent years using several sorption techniques. However, due to the volatile nature of these compounds, it is difficult to adhere them on a polymeric matrix [7]. Likewise, it is difficult to adsorb essential oils on polymeric surfaces such as polypropylene (PP), polystyrene (PS), low density polyethylene (LDPE) and linear low density polyethylene (LDPE) due to their hydrophobic nature [8]. The aim of this work is to functionalize LDPE and PS surfaces using UV light in order to create hydrophilic surfaces to adhere OEO on them as well as to evaluate their antimicrobial properties, cytotoxicity and mechanical properties.

MATERIALS AND METHODS

LDPE and PS commercial polymers were cut in pieces of 5x2 cm and 5x5 cm. UV light treatment was carried out during 60 min using a 254 nm wavelength UV lamp, room temperature, 1 atmosphere of pressure and 5 cm distance between the lamp and the sample [9, 10].

OEO (Lippia berlandieri Schauer, 67.3 % thymol, 31.3 % carvacrol, limonene 1.4 % and cymene 0.2 %) was obtained through steam distillation according to Hernández and co-workers [11]. The polymeric samples were immersed in OEO at different concentrations (0.5, 1.0, 1.5 % v/v) during 3 h as reported by Mauriello and co-workers [12]. Finally, the films were dried overnight at room temperature.

A chemical analysis was carried out after incorporation of the AEO on the polymer surface, using a Nicolet 6700 FTIR equipment. A range of 600-4000 cm^{-1} wavelengths was used.

Contact angles were determined using a Kernko G-1 goniometer (1-180° ± 0.1°). A 5 µL droplet of filtered, deionized distilled water was placed on the surface of the samples at room temperature. After 1 min, the contact angles were measured using a video capture software (TS Viewer).

Mechanical properties were determined using an Instron Universal machine. The tensile stress analysis was carried out for the PS samples at a temperature of 25 °C and a deformation speed of 1 mm/s. The flexural analysis was carried out for the LDPE samples at a temperature of 25 °C and a deformation speed of 5 mm/min. In both cases a 2,000 N load cell was used.

The antimicrobial activity of the samples was carried out by the turbidimetric method [9] using bacterial strains of *Escherichia coli (E. coli)* and *Micrococcus lysodeikticus (M. lysodeikticus)*, using cells alone (without being exposed to any material) and samples of LDPE and PS without treatment (antimicrobial agent) as controls. 1 mL of inoculum was added to each sample and incubated at 37 °C for 24 h. Subsequently, a reading of 495 nm was taken in a Bio-Rad device, Benchmark Microplate Reader. The antibacterial activity (or percentage of inhibition) was calculated taking into account the optical density according to equation (1):

$$\%\ inhibition = \frac{Bacterial\ control\ average}{Sample\ average}\ x\ 100 \quad (1)$$

All samples and controls (polymer without OEO and cells without material) were seeded with fibroblast line 3T3. 1.5 x 104 cells during 24 and 72 h, in D-MEM supplemented with fetal bovine serum (FBS) and penicillin-streptomycin. The viability was determined using 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay according to Valencia-Gómez and co-workers [13]. At the end of the incubation period, the culture medium was discarded and 225 µl of D-MEM solution, 1 % Antibiotic and 10 % FBS and 25 µl of MTT (5 mg / ml of PBS 1X) were added. A blank was created composed only of D-MEM, 1 % Antibiotic and 10 % SFB and MTT. Subsequently, they were incubated for a period of 1 hour at 37 ° C. Once the time elapsed the medium was discarded and DMSO was added to solubilize formazan crystals. Subsequently absorbance reading of the solution was taken in a microplate reader at 570 nm (Bio-Rad, Benchmark Microplate Reader). All measurements were carried out in triplicate. Samples mean were compared through t-student test ($P<0.05$).

RESULTS AND DISCUSSION

Polymers vibrational analysis

UV radiation exhibited significant changes on the LDPE and PS polymers in different regions of the infrared spectrum as can be seen in Fig 1 and Fig 2 respectively. The occurrence of absorption bands at 1643 cm^{-1}, and 3170 cm^{-1} in LDPE polymer are the characteristic of C=N double bonds and a NH bond respectively, they could be associated to possible contamination with atmospheric gases (spectra b and d). An increased absorption band at 3350 cm^{-1} was observed (spectra b and d), which is related to hydroxyl groups that occur due to the oxidation of the polymeric surface caused by metastable oxygen species generated by the UV radiation as reported by Moyano [14].

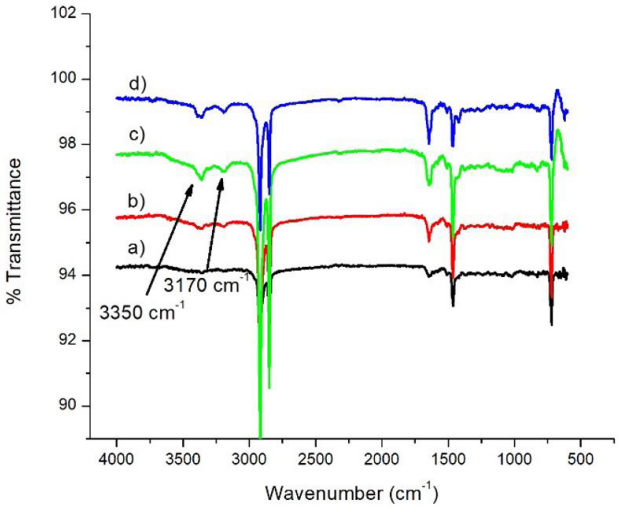

Fig. 1: LDPE spectrum (a) before UV treatment (b) after 30 min irradiation (c) after 45 min irradiation (d) after 60 min irradiation.

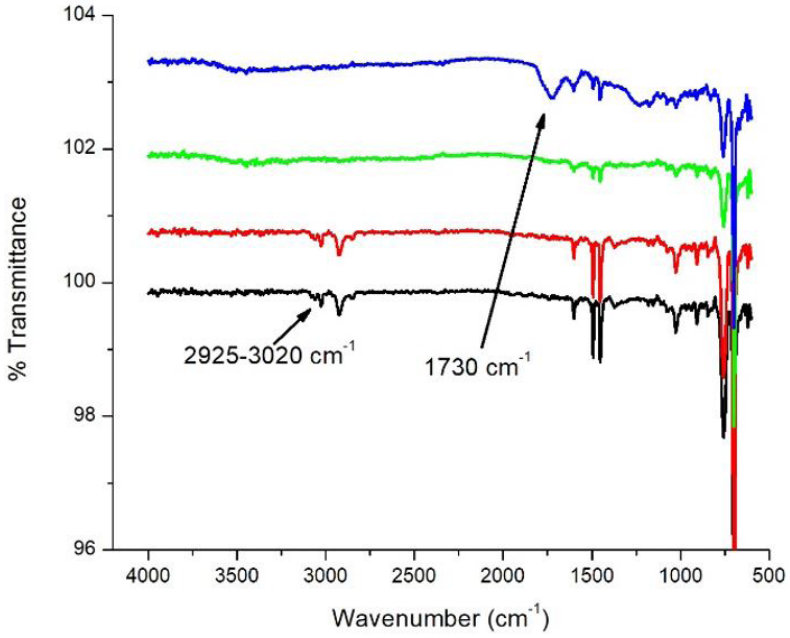

Fig. 2: PS spectrum (a) before UV treatment (b) after 30 min irradiation (c) after 45 min irradiation (d) after 60 min irradiation.

Figure 2 shows the infrared spectrum of PS, where absorption bands at 1435 and 1480 cm^{-1} are observed, which correspond to CH bonds of methylene groups. These bands decrease in intensity as the exposure time to UV light increases, as described by Bermudez and Salazar [15]. The absorption bands of the PS in the regions of 2925 and 3020 cm^{-1} that correspond to the inherent stretching of CH bonds disappeared due to the oxidation of the polymer. Prolonged PS exposure to UV radiation (Figure 2, c and d) generated an absorption brand in the 1730 cm^{-1} region corresponding to C=O stretching bonds that represents an aldehyde group on the surface of the polymer [16].

Determination of the contact angle

The polar functional groups, formed due to polymer´s surface oxidation, allowed the significant decrease of contact angle measured with water and OEO 1.5 % v/v (Table 1). It was observed that the wettability by OEO is higher compared to water; since highly polar molecules such as water are less attracted by non-polar molecules of the LDPE or PS. It was found that, as the time of UV light exposure of the polymer´s surface increased, the contact angle of the water on LDPE decreased, with mean values from 75.33º to 53.62º, after 60 min (figure 3). At this time, polar groups became evident on the FTIR, previously analyzed, which represent anchor points to polar molecules on the polymeric surfaces [17].

The mean value of the contact angle on untreated PS was 78.37º and experienced a considerable decrease to 55.25º, after 60 min of UV light exposure (figure 4). The formation of oxidized chemical species through chemical photo-oxidation

Table 1. Mean values of contact angles (°) of water and OEO on polymers after treatment with UV light for 0–60 min.

Liquid	Material	Untreated	30 min UV	45 min UV	60 min UV
Water	LDPE	75.33 ± 5.76	75.56 ± 1.87	74.01 ± 0.11	53.62 ± 2.70*
	PS	78.37 ± 1.47	57.04 ± 2.25	80.08 ± 3.63	55.25 ± 1.33*
OEO	LDPE	54.54 ± 0.94	50.61 ± 1.02	57.20 ± 1.26	37.77 ± 1.12*
	PS	33.98 ± 4.16	33.74 ±4.42	32.78 ± 0.06	30.23 ± 0.49*

Reported values are means ± standar deviation. * indicates significant differences ($p \leq 0.05$)

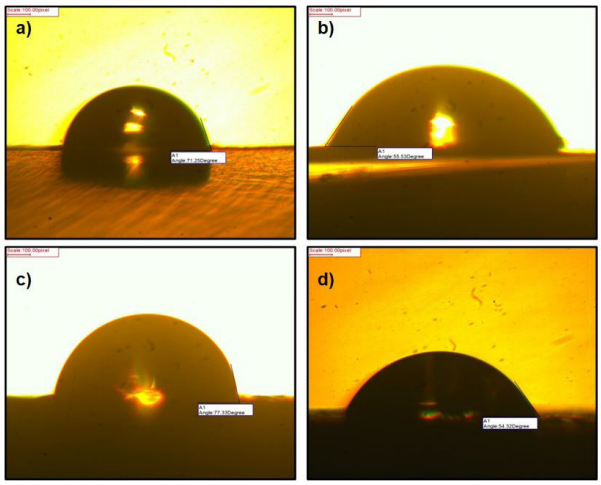

Fig. 3: Examples of water contact angles on polymeric surfaces: a) LDPE untreated (θ=71.25°); b) LDPE after 60 min exposure to UV light (θ=55.53°); c) PS untreated (θ=77.33°); d) PS after 60 min exposure to UV light (θ=54.32°).

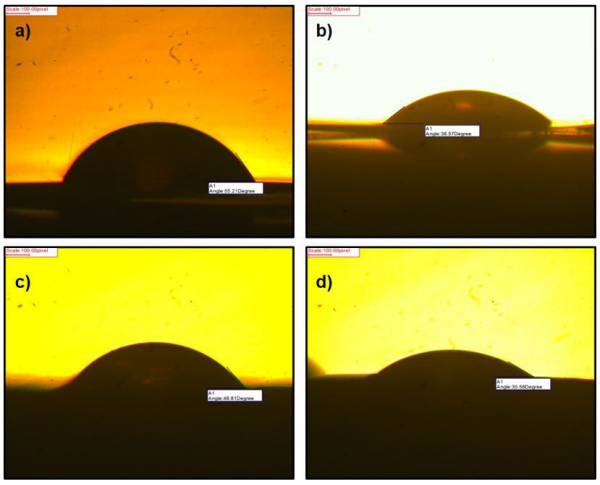

Fig. 4: Examples of OEO contact angles on polymeric surface: a) LDPE untreated (θ=54.54°); b) LDPE after 60 min exposure to UV light (θ=37.77°); c) PS untreated (θ=33.98°); d) PS after 60 min exposure to UV light (θ=30.58°).

reactions, such as carbonyl (C=O), carboxyl (COOH) or hydroxyl (OH) groups on LDPE and PS are responsible of the significant decrease of the measured contact angles with water and OEO.

It was not possible to measure the contact angle using the concentrated OEO directly on the polymeric surface because it uneven spreads over the non-polar surface of the polymers due to its hydrophobic nature. The use of polysorbate 20 as an emulsifier allowed to stabilize the concentration of the OEO in water and consequently was possible to measure its wettability on the LDPE and PS. The values of the contact angle decrease as that the UV light exposure time was increased. These mean values were 54.54° for LDPE without treatment and 37.77° after 60 min of exposure (optimal exposure time over 30 and 45 min, respectively), while for the PS, mean values of 33.98° were obtained without treatment and 30.23° after 60 min treatment of UV light as shown in Table 1 and Figure 4.

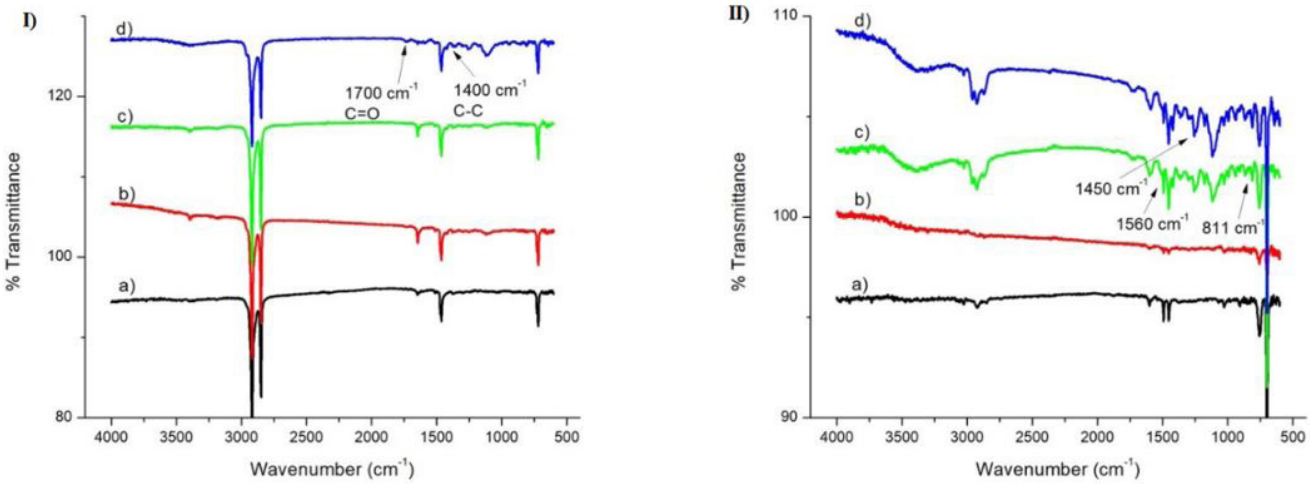

Fig. 5: IR spectra for the interaction I) OEO-LDPE and II) OEO-PS at different concentrations; a) control (polymer without OEO), b) 0.5 % OEO; c) 1.0 % OEO and d) 1.5 OEO.

Interaction polymer-OEO

The FTIR analysis at 1.5 % concentration, exhibits characteristics bands for thymol and carvacrol, as shown in figure 5I. Absorption bands at 1400cm^{-1} are associated to C-C bound of aromatic rings, related to carvacrol. Another band at 811 cm^{-1} corresponds to the presence of C-H bonds, and one more at 1460 cm^{-1} is characteristic of OH functional groups. The absorption band at 1700 cm^{-1}, is associated to carbonyl groups and may occur due to the interaction between LDPE with OEO by weak bonds such as hydrogen bonds, Figure 5I-a [18]. On the other side, bands at 811 cm^{-1} and 947 cm^{-1} were observed i n the IR spectra of PS (Fig. 5II-b). These absorption bands represent the formation of CC bonds and OH groups attributed to the formation of a hydroxyl group (Fig 5II, c and d) that are characteristic of thymol [19]. The absorption bands in the 1410-1450 cm^{-1} (LDPE) and 1560 cm^{-1} and 1585 cm^{-1} (PS) ranges are due to the presence of aromatic rings with stretching CC bonds [20].

There is limited information about the OEO-LDPE and OEO-PS interaction model. In the present work, it is proposed that the interaction can occur through weak bonds by hydrogen bridges on the surface of the polymer. Firstly, the short-wave UV light modifies the LDPE and PS surface generating polar groups, which may function as an anchor point with the OH groups of the OEO. Then, the bond is given by attracting hydrogen from the hydroxyl group at the end of the carvacrol (or thymol) molecule and a pair of non-shared electrons in the generated carbonyl group.

Table 2: Optical density data after 24 and 72 h of incubation in contact with polymers at different concentrations of OEO.

Material	24 h		72 h	
	Mean	% viability	Mean	% viability
LDPE				
Cellular control	2.87 ± 0.12	100	3.30 ± 0.12	100
LDPE control	2.94 ± 0.00	86.79	5.61 ± 0.02	170.31
0.5 % OEO LDPE	3.11 ± 0.26	108.17	2.53 ± 0.41	76.72
1.0 % OEO LDPE	0.38 ± 0.02	13.16*	0.35 ± 0.05	10.4*
1.5 % OEO LDPE	0.36 ± 0.03	12.41*	1.08 ± 0.19	32.8*
PS				
Cellular control	2.87 ± 0.12	100	3.3 ± 0.12	100
PS control	1.89 ± 0.00	64.62*	5.15 ± 0.02	156.27
0.5 % OEO PS	3.04 ± 0.12	105.84	2.94 ± 0.38	88.87
1.0 % OEO PS	0.48 ± 0.04	16.67*	0.33 ± 0.33	9.89*
1.5 % OEO PS	0.46 ± 0	15.91*	0.34 ± 0.34	10.39*

Reported values are means ± standar deviation. * indicate significant differences respect control ($p \leq 0.05$)

Cytotoxicity assay

Cells exposed to the control polymer, LDPE (without OEO), showed 13.21 % cell death after 24 h, whereas at 72 h there was a positive growth of 170.31 % as shown in table 2. The concentration of OEO at which the cells begin to die is from 1.0 % where values below 70 % of cell viability were obtained. This is considered as cytotoxic according to the in vitro toxicity test of the international standard for the evaluation of medical instruments [21]. It is possible that at high concentrations of OEO the cytotoxic effect is due to reactive oxygen species that lead to cell membrane rupture [22]. Such cytotoxic mechanisms include oxidative stress, cell membrane rupture, and the presence of oxygen reactive species [23]. This effect is similar in PS where it was observed that the cells are not affected by the control polymer and even develop, after 72 h. however from a concentration of 1.0 % OEO there is a cytotoxic effect after 24 h and 72 h, respectively.

Images observed under microscope at 20x magnification, show the fibroblast proliferation on the OEO-LDPE conjugate at a concentration of 0.5 %. It was observed that the fibroblast proliferated and adhered throughout the polymer area after 24 h. After 72 h (figure 6, subsection b) a great cell dispersion, as well as a greater activity of filopodia (Cytoplasmic projection) is observed. At these conditions fibroblasts showed extended form, spindles or half-moon like with a round nucleus. Cells exhibited a confluent monolayer, as well as the presence of extracellular matrix (ECM) and cytoplasmic projections that connected adjacent cells [24]. It was found that the fibroblast culture, presented cellular damage at membrane level when it was in contact with 1.0 % OEO-LDPE , after 24 and 72 h (figure 6 c). Concentration of 1.5 % exhibited cytotoxic effect on fibroblasts, evidenced through damage on its outer membrane and granular particles in the center after 24 h of incubation, as well as poor or non-presence of cells after 72 h.

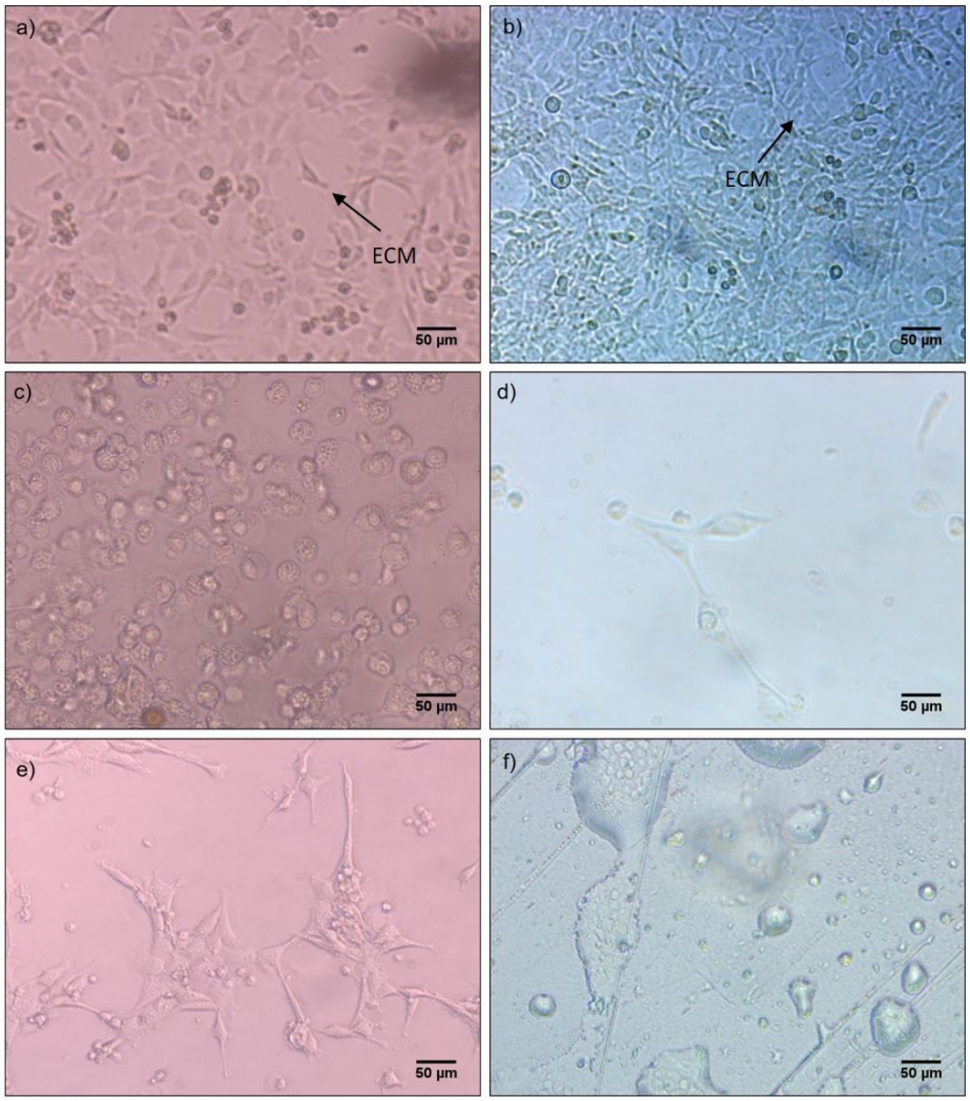

Fig. 6: Fibroblast micrographic on LDPE surface at different concentrations of OEO at 24 and 72 h. a) 0.5 % OEO at 24 h; b) 0.5 % OEO at 72 h; c) 1.0 % OEO at 24 h; d) 1.0 % OEO at 72 h; e) 1.5 % OEO at 24 h; f) 1.5% OEO at 72 h.

Table 3. Mean inhibition percentage of polymeric films added with different concentratiosn of OE against M. lysodeikticus and E. coli.

Material	*M. lysodeikticus*		*E. coli*	
	Mean	Activity %	Mean	Activity %
LDPE				
LDPE control	1.50 ± 0.02	1.30	1.18 ± 0.02	1.00
0.5 % OEO LDPE	1.49 ± 0	6.41	1.10 ± 0.02	7.53*
1.0 % OEO LDPE	1.48 ± 0.02	8.16*	1.22 ± 0.03	4.21*
1.5 % OEO LDPE	1.46 ± 0.03	10.71*	1.09 ± 0.02	8.00*
PS control				
PS control	1.01 ± 0.02	1	1.17 ± 0.02	1.80
0.5 % OEO PS	1.45 ± 0.06	4.80	1.12 ± 0.02	6.00
1.0 % OEO PS	1.39 ± 0.08	8.66*	1.07 ± 0.03	10.00*
1.5 % OEO PS	1.29 ± 0.03	15.42*	1.04 ± 0.02	12.40*

Reported values are means ± standar deviation. * indicate significant differences respect control ($p \leq 0.05$)

Determination of the films antibacterial activity

Table 3 shows the antibacterial tests results on two selected bacteria by turbidimetry. Both LDPE and PS control, showed an inhibitory capacity, inferior to 1.5 % for each of the selected bacteria; but as the antimicrobial agent concentration added on the polymeric film increased, the bactericidal activity augmented as well. For instance, LDPE at 0.5 % showed 7.53 % of inhibition, while at a concentration of 1.5 % an 8 % inhibition was obtained. For PS the highest inhibition percentage was 12 % at a concentration of 1.5 % of OEO, and the minimum was of 6 % at a concentration of 0.5 %. See table 3

In the present assay, *M. Lysodeikticus* exhibited greater susceptibility against the phenolic compounds contained on the OEO than *E.coli.* Several researches mention this effect [1]. They reported greater inhibition zones against *Staphylococcus aureus,* a gram positive bacteria, in comparison with *E.coli*, a gram negative bacteria, exposed to OEO at a concentration of 2.0 %. The higher efficiency against Gram positive bacteria is attributed to a direct interaction of the essential oil on the hydrophobic components of the cellular membrane, due to the hydrophobic nature of the OEO that penetrates the hydrophilic membrane of Gram negative bacteria (low lipid content), which makes them more resistant to essential oils [25].

Table 4: Mean values for LDPE films' mechanical properties at different concentrarions of OEO ($p<0.05$)

Material	Stress – strain (MPa)	Young Module (MPa)	Maximum effort (MPa)	% of deformation	Maximun Deformation (%)
LDPE control	12.07 ± 2.35	29.25 ± 0.35	17.45 ± 0.18	353 ± 2.03	705 ± 0
0.5 % OEO LDPE	11.95 ± 2.50	32.62 ± 3.71	17.22 ± 0.33	202 ± 2.01	687 ± 0
1.0 % OEO LDPE	11.93 ± 2.70	28.94 ± 1.49	17.40 ± 0.65	368 ± 2.12	708 ± 0
1.5 % OEO LDPE	11.74 ± 2.45	27.52 ± 2.79	16.24 ± 0.61	355 ± 2.05	664 ± 0

Reported values are means ± standar deviation.

Mechanical properties

Table 4 shows the results for tensile stress (TS), Young modulus (EM) and stress-strain (%E) of the LDPE films functionalized with OEO. No significant differences were found among them, despite the gradual increase in OEO concentration (Figure 7 and table 4). The OEO addition, even at 1.5 % v/v concentration, did not affect the polymers intramolecular forces neither their flexibility.

Three-point flexural test using specimens of the same size and varying the OEO concentration in (0, 0.5, 1.0 and 1.5) % v/v were performed. The load-displacement data performed in PS were recorded in Table 5. The plot of PS data at concentrations of 0 % and 0.5 % appears to be divided into three sections. The first section is the linear slope segment before reaching the maximum stress point, which represents the stiffness of the sample (Fig. 8).

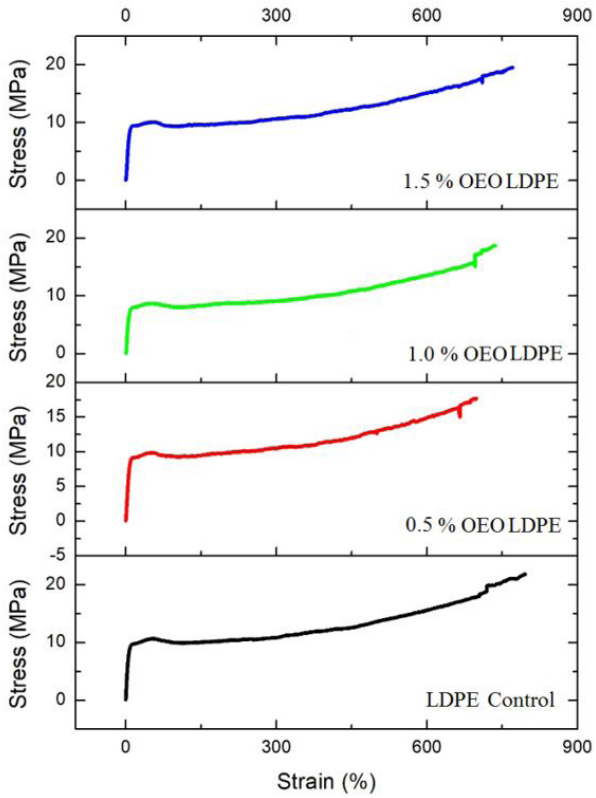

Fig. 7: Graph for LDPE Films' stress strain at different concentrations of OEO

Table 5: Mean values of bending efforts (bending) for PS polymeric matrixes at different concentrations of AEO.

Property/sample	PS control	0.5 % OEO PS	1.0 % OEO PS	1.5 % OEO PS
Maximum effort (MPa)	5.51 ± 0.65	5.20 ± 0.65	2.60 ± 0.65	3.26 ± 0.11
Maximum strain (%)	0.052 ± 0.007	0.06 ± 0.004	0.035 ± 0	0.045 ± 0.011

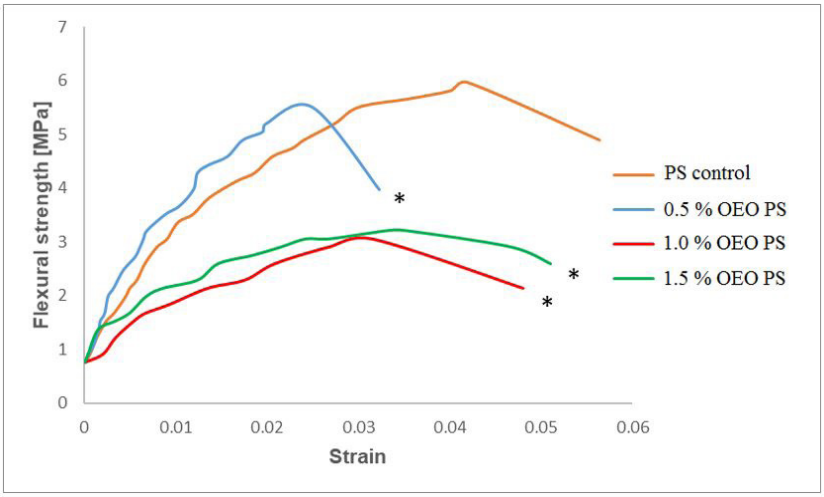

Fig 8: Bending stress strains for PS at different concentrations of OEO, having 0% OEO as control sample
** Significant differences with respect to control ($p < 0.05$ for each sample).*

According to Tang and co-workers [26], the second region where the graph reaches the maximum stress point is due to the growth of a fracture zone. In this work, the increase in OEO concentration is likely to be directly related to the amount of growth of micro-fractures before the maximum point. The third region corresponds to the segment of material rupture. It was observed that an increase in concentration of 1.0 and 1.5% of OEO decreased the tensile stress compared to the control sample, causing a significant loss of resistance of the material to the deformation under a load.

CONCLUSION

Low density polyethylene and polystyrene were functionalized with oregano´s essential oils (OEO). UV light exposure treatment generated polar groups that allowed the polymers functionalization with OEO. Functionalized LDPE and PS (0.5, 1 and 1.5 % v/v OEO) caused significant damage on Gram positive or Gram negative bacteria. Mechanical properties of LDPE were not affected by the OEO additions. PS mechanical properties were diminished by OEO in concentrations from 1 to 1.5% % v/v. LDPE and PS functionalized with 1.5 % v/v OEO showed cytotoxicity according to MTT assay, damage on their cytoplasmic membrane of the cells exposed to this material was observed.

REFERENCES

[1] A. Aguirre, R. Borneo, A. León, "Antimicrobial, mechanical and barrier properties of triticale protein films incorporated with oregano essential oil". Food Bioscience, 1, 2-9, 2013.

[2] R. Coles, D. McDowell, M. Kirwan, " Food Packaging Technology". London: Wiley-Blackwell, 2009.

[3] M. L. Faleiro, "The mode of antibacterial action of essential oils. Science Against Microbial Pathogens". Communicating Current Research and Technological Advances, 2, 1143-1156, 2011.

[4] S. Burt, "Essential oils: Their antibacterial properties and potential applications in foods. A review". International Journal of Food Microbiology, 94, 223–253, 2004.

[5] T. R. Callaway, J. A. Carroll, J. D. Arthington, T. S. Edrington, R. C. Anderson, S. C. Ricke, "Citrus products and their use against bacteria: potential health and cost benefits. Nutrients, dietary supplements, and nutriceuticals: Cost analysis versus clinical benefits", 1st. ed. New York: Humana Press, 2011.

[6] HY. Gong, WH. Liu, X. Zhou, "Analysis of essential oils of Origanum vulgare from six production areas of China and Pakistan", Revista Brasileira de Farmacognosia, 24, 25-32, 2014.

[7] C. Pérez, C. Regalado,C. Rodríguez, J. Barbosa, F. Villaseñor, "Incorporation of antimicrobial agents in food packaging films and coatings", Advances in Agricultural and Food Biotechnology, 2, 193-216, 2006.

[8] E. Torlak, M. Nizamlioglu, "Antimicrobial effectiveness of chitosan-essential oil coated plastic films against foodborne pathogens", Journal of Plastic Film & Sheeting, 27(3), 235–248, 2001.

[9] M. Moyano,and J. Martínez, "Surface treatment with UV-ozone to improve adhesion of vulcanized rubber formulated with an excess of processing oil", International Journal of Adhesion and Adhesives, 55, 106–113, 2014.

[10] S. K. Øiseth, A. Krozer,J. Lausmaa, B. Kasemo, Ultraviolet Light Treatment of Thin High-Density Polyethylene Films Monitored with a Quartz Crystal Microbalance. Polymer Surface Modification: Relevance to Adhesion, 92, 2833–2839, 2004.

[11] M. Hernández-González, C. P. Berumen, H. S. Ruíz, C. R. Salazar, J. H. Paz, I. Olivas-Armendáriz, C. R. González, "Polysuccinimide functionalized with oregano's essential oil extracts, an antimicrobial extended release bio-material", Materials Letters, 191, 73-76, 2017.

[12] G. Mauriello, D. Ercolini, A. La Storia, A. Casaburi, F. Villani, "Development of polythene films for food packaging activated with an antilisterial bacteriocin from Lactobacillus curvatus 32Y", Journal of Applied Microbiology, 97 (2), 314–322, 2004.

[13] R. Banjara, S. Jadhav, S. Bhoite, "Antibacterial activity of di-2-ethylaniline phosphate screened by paper disc diffusion method", Journal of Applied Pharmaceutical Science, 2, 230-233, 2012.

[14] L. E. Valencia-Gómez, S. A. Martel-Estrada, C. Vargas-Requena, J. L. Rivera-Armenta, N. Alba-Baena, C. Rodríguez-González, I. Olivas-Armendáriz, "Chitosan/ Mimosa tenuiflora films as potential cellular patch for skin regeneration", International journal of biological macromolecules, 93, 1217-1225, 2016.

[15] L. Bermúdez, and R. Salazar, "Synthesis and characterization of the polystyrene – asphaltene graft copolymer by ft-ir spectroscopy", CT&F - Ciencia, Tecnología y Futuro, 3(4), 157-167, 2008.

[16] R. M. Silverstein, G. C. Bassler, T. C. Morrill, "Spectrometric Identification of Organic Compounds", 7 ed. New York: John Wiley and Sons, 2005.

[17] H. Kaczmarek, J. Kowalonek, A. Szalla, A. Sionkowska, "Surface modification of thin polymeric films by air-plasma or UV-irradiation", Surface Science, 507, 883–888, 2002.

[18] A. Schulz, Z. Gülcan, A. Baranska, A. Krüger, D. Musa Özcan, "Characterization of essential oil plants from Turkey by IR and Raman spectroscopy", Vibrational Spectroscopy, 39, 249–256, 2005.

[19] I. S. Al-Sheibany, " Isolation and Identification of Volatile oils from Iraqi Thyme (Thymbra Spicata) and study the antimicrobial activity", National Journal of Chemistry, 18, 289-298, 2005.

[20] J. Gulmine, P. Janissek, H. Heise, L. Akcelrud, "Polyethylene characterization by FTIR", Polymer Testing, 21, 557–563, 2002.

[21] ISO 10993-5 (1999). Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity. International Organization for Standardization. 1-8.

[22] M. Akhtar, M. Ahamed, M. Fareed, S. Alrokayan, S. Kumar, "Protective effect of sulphoraphane against oxidative stress mediated toxicity induced by CuO nanoparticles in mouse embryonic fibroblasts BALB 3T3", The Journal of Toxicological Sciences, 37, 139-148, 2012.

[23] N. Jojić, V. Kojić, D. Kojić, K. Stankov, G. Bogdanović, "Cytotoxicity of single-walled carbon nanotubes to human lung carcinoma cells: the influence of N-acetylcysteine", Archive of oncology, 21(2), 59-61, 2013.

[24] S. Kim, H. Ha, Y. Ko, S. Yoon, J. Rhee, M. Kim, H. Lee, G. Khang, "Correlation of proliferation, morphology and biological responses of fibroblasts on LDPE with different surface wettability", Journal of Biomaterials Science, Polymer Edition, 18 (5), 609–622, 2007.

[25] J. Calo, P. Grandall, C. O'Bryan, S. Ricke, "Essential oils as antimicrobials in food systems: A review", Food Control, 54, 111-119, 2015.

[26] W. Tang, H. Cui, S. Tahmasbi, "Fracture Properties of Polystyrene Aggregate Concrete after Exposure to High Temperatures", Materials, 9, 630, 2006.